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Research Paper

Topical iontophoretic delivery of ionizable, biolabile aciclovir prodrugs: A rational approach to improve cutaneous bioavailability

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ABSTRACT

The objective was to investigate the topical iontophoretic delivery of a series of amino acid ester prodrugs of aciclovir (ACV-X, where ACV = aciclovir and X = Arg, Gly, Ile, Phe, Trp and Val) as a means to enhance cutaneous delivery of ACV. The newly synthesized prodrugs were characterized by ^{1}H NMR and high resolution mass spectrometry. Analytical methods using HPLC–UV were developed for their quantification and each method was validated. Investigation of solution stability as a function of pH showed that all ACV-X prodrugs were relatively stable in acid conditions at pH 2.0 and pH 5.5 for up to 8 h but susceptible to extensive hydrolysis at pH 7.4 and under alkaline conditions (pH 10). No ACV-X hydrolysis was observed after contact for 2 h with the external surface of porcine stratum corneum. However, there was significant hydrolysis following contact with the dermal surface of dermatomed porcine skin, in particular, for ACV-Arg. Passive transport of ACV and ACV-X prodrugs from aqueous solution after 2 h was below the limit of detection. Iontophoresis of ACV at 0.5 mA/cm^2 for 2 h led to modest ACV skin deposition ($Q_{DEP,ACV}$) of 4.6 ± 0.3 nmol/cm^2. In contrast, iontophoresis of ACV-X prodrugs under the same conditions produced order of magnitude increases in cutaneous deposition of ACV species, that is, $Q_{DEP,TOTAL} = Q_{DEP,ACV} + Q_{DEP,ACV\text{-}X}$. $Q_{DEP,TOTAL}$ for ACV-Gly, ACV-Val, ACV-Ile, ACV-Phe, ACV-Trp and ACV-Arg was 412.8 ± 44.0, 358.8 ± 66.8, 434.1 ± 68.2, 249.8 ± 81.4, 156.1 ± 76.3, 785.9 ± 78.1 nmol/cm^2, respectively. The extent of bioconversion of ACV-X to ACV in the skin was high and the proportion of ACV present ranged from 81% to 100%. The skin retention ratio, a measure of the selectivity of ACV species for deposition over permeation after iontophoretic delivery of ACV-X prodrugs, was dependent on both the rate of transport and the susceptibility to hydrolysis of the prodrugs. Skin deposition of ACV and its six prodrugs were investigated further as a function of current density (0.125, 0.25 and 0.5 mA/cm^2); the effect of duration of current application (5, 10, 30, 60 and 120 min) was evaluated using ACV-Arg and ACV-Ile. Iontophoresis of ACV-Arg and ACV-Ile at 0.25 mA/cm^2 for only 5 min resulted in the deposition of appreciable amounts of ACV (36.4 ± 5.7 nmol/cm^2 and 40.3 ± 6.1 nmol/cm^2, respectively), corresponding to supra-therapeutic average concentrations in skin against HSV-1 or HSV-2. The results demonstrated that cutaneous bioavailability of ACV could be significantly improved after short-duration iontophoresis of ionizable, biolabile ACV-X prodrugs.

1. Introduction

Aciclovir (ACV, 9-[(2-hydroxyethoxy)methyl])-9H-guanine, Zovirax®) is commonly used to treat *herpes labialis*, but its poor and variable oral bioavailability (10–20%) decreases efficacy. This has led to the development of valaciclovir (VCV, Valtrex®), the L-valyl ester of aciclovir, which is a substrate for the mammalian intestinal peptide transporters, PEPT1 and PEPT2 [1]. Although this displays higher oral bioavailability (30–50%) [2], gram amounts of VCV have to be administered orally (1000 mg three times a day) to treat an essentially local disorder. Efficient topical ACV delivery should enable targeted therapy, reduce circulating drug levels and attenuate the risk of renal insufficiency [3].

However, despite the therapeutic rationale and high specific activity against HSV-1 and HSV-2 in virus-infected cells, clinical studies of several topical ACV formulations against *herpes labialis* have given mixed results. Although a reduction in healing time was reported with 5% ACV cream [4], clinical failures were also seen [5]. Indeed, ACV ointment (5%) seemed to have less or even no clinical benefit [6,7]. Successful topical treatment relies on effective ACV delivery through intact skin since the epidermal

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pathology in the early stages of viral infection does not alter stratum corneum permeability [6]. Drug penetration must be sufficient to achieve therapeutic concentrations in the basal epidermis, where the virus is found. Furthermore, drug therapy should be initiated as early as possible, e.g. in the prodrome or erythematous lesion stages [7–9]. Initiation of therapy more than 12 h after the appearance of symptoms, when the classical lesions such as papule, vesicle, ulcer or crust have been established, was observed to be ineffective and did not provide any benefit over the natural resolution of the infection [7,9,10].

Effective treatment of *herpes labialis* needs early intervention and rapid delivery of sufficient drug to not only shorten the duration of the episode [9] but also to decrease virus entry into sensory neurons and the copy number of latent genome cloned. A smaller reservoir of latent virus would decrease its ability to reactivate periodically from latency [11]. However, ACV is not a good candidate for passive topical delivery. Its polarity ($\log D_{pH7} = -1.76$) and sparing solubility in both aqueous and lipid media render its formulation difficult and limit its partitioning into the stratum corneum. A slow rate of delivery and poor bioavailability result in delayed antiviral intervention in the basal epidermis and sub-inhibitory concentrations which translate into poor clinical efficacy [12].

Iontophoresis is a technique that involves the application of a mild electric current and can be used to substantially increase the transport of molecules into and across the skin [13,14]. It is best-suited to water soluble, charged molecules and ACV (pK_{a1} 2.27; pK_{a2} 9.25) is uncharged under physiologically acceptable conditions and has poor aqueous solubility, ~0.2% at 25 °C [15] – thus, it is far from being an ideal candidate for iontophoresis. Although cutaneous delivery of therapeutic amounts of ACV by iontophoresis has been reported, it could only be achieved with formulations at pH 3 or 11 [16,17], which are unsuitable for human use. In contrast, we have previously shown that anodal iontophoresis of VCV, with a formulation pH of 5.24 or 5.65, was able to produce order of magnitude increases in ACV delivery [18]. Iontophoretic transport was facilitated by the positively charged valyl group, which was then cleaved by esterases to release ACV and valine during cutaneous transport.

The objective of the project was to investigate the electrotransport of a series of amino acid ester prodrugs of ACV (ACV-X, where X = Arg, Gly, Ile, Phe, Trp and Val) and to determine the influence of the different amino acid components on ACV delivery. The prodrug strategy has been widely used to develop topical and transdermal formulations – most routinely in the case of corticosteroid esters. However, in the case of passive administration, the objective is to increase molecular lipophilicity, for example, by masking hydroxyl groups at the 16α and 17α positions through acetonide formation (e.g. triamcinolone acetonide) or through esterification of the hydroxyl groups at the 17α and 21 positions with short chain carboxylic acids (e.g. betamethasone dipropionate) (Fig. 1a). Conversely, in the case of these "iontophoretic" prodrugs, the aim was to introduce polar ionizable moieties that increase hydrophilicity, aqueous solubility and most importantly, impart a charge to the molecule enabling it to electromigrate into the skin and so benefit more efficiently from iontophoresis (Fig. 1b). In this case, the hypothesis was that the presence of the positively charged moieties in the ACV-X prodrugs would improve their aqueous solubility and facilitate their electromigration into the skin, where ACV would be released following enzymatic cleavage by cutaneous esterases; this would enable greater amounts of ACV to be delivered more rapidly to the skin (Fig. 1c).

The specific aims of this study were (i) to synthesize, purify and characterize the amino acid ester prodrugs, (ii) to develop and validate robust HPLC–UV methods to quantify ACV and each prodrug simultaneously, (iii) to determine the stability of the prodrugs in aqueous solution as a function of pH and in the presence of skin, (iv) to compare passive and iontophoretic transport by measuring skin deposition and cumulative permeation of ACV species (that is, ACV and each ACV-X prodrug) and to evaluate skin retention and total bioconversion ratios, and (v) to optimize iontophoretic conditions for milder and shorter current application.

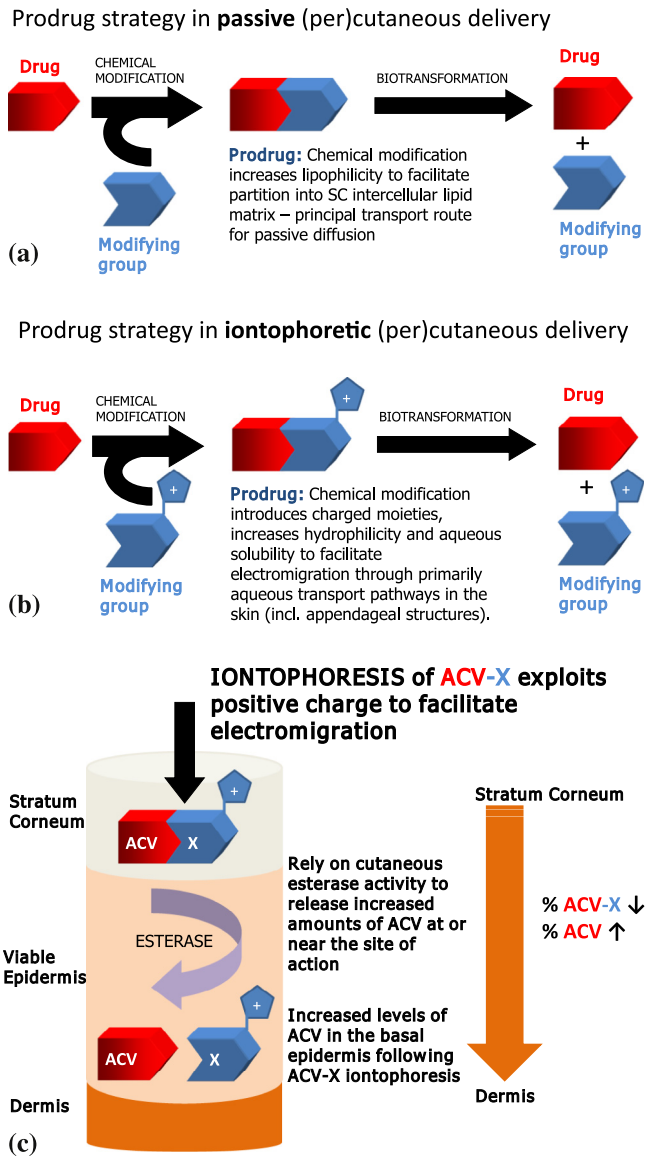

Fig. 1. Schematic representations illustrating the different rationale for using prodrugs to improve (a) passive and (b) iontophoretic transport into and across the skin. (c) Water soluble amino acid ester prodrugs of aciclovir (ACV-X) containing ionized moieties benefit from electromigration as the principal transport mechanism. They are hydrolyzed in the skin to release ACV and the amino acid. The rate of transport and the rate of hydrolyze influence the biodistribution of the prodrug and the relative amounts permeated and retained within the membrane.

2. Materials and methods

2.1. Materials

ACV was purchased from Sequoia Research Products Ltd. (Pangbourne, UK). *N,N′*-Diisopropylcarbodiimide (DIC) was purchased from TCI (Tokyo, Japan). Boc-Gly-OH, Boc-Ile-OH, Boc-Trp-OH, Boc-Val-OH and 1-hydroxybenzotriazole hydrate (HOBt) were supplied by Sigma–Aldrich (Buchs, Switzerland).